Protocol 0107—488
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Title: A Phase 1a, randomized study of escalating single doses of VRX496 in subjects with AIDS

1. The advantages of using HIV vectors instead of MLV vectors in this trial include (1) co-localization of the vector RNA with wt-RNA may be more effective to inhibit the wt-RNA function; (2) competition between the vector and the wt HIV for transactivators important for replication; (3) HIV vectors are more efficient to transduce human T cells. The investigators have already indicated in the application that the current vector, just by itself without the antisense env gene, did not seem to interfere with wt HIV replication. This is consistent with observations reported by others (for example, Evans & Garcia, Hum Gene Ther 11:2331-2339, An et al., J Virol. 73: 7671-7677). The advantage of RNA co-localization remains hypothetical at this point since MLV vectors containing the antisense env gene has also been shown to effectively block wt HIV replication in culture (Veres et al. J. Virol. 72: 1894-1901). While close to 100% transduction efficiency of CD4+ lymphocytes with HIV vectors is stunning, can the similar transduction efficiency be obtained consistently with the lymphocytes from HIV patients? Has the same protocol for lymphocytes stimulation and vector transduction been tested with MLV vectors? If MLV can achieve similar levels of transduction, the risks of using HIV vectors may not justify this application.

2. One major concern is related to the helper construct. In this case, all the genes required for the generation of an infectious vector are included in a single plasmid. Such a design seems to increase the risk of RCR generation through recombination during transfection or co-packaging of vector and helper RNAs. It is not clear whether such an approach enhances vector titers since only two plasmids are required for vector production in co-transfection. What is the crude infectious titers from such an approach ? Currently, the most widely used system for vector production involves several plasmids: a gag-pol expression plasmid, a VSV-G expression plasmid, a Rev expression plasmid and the vector construct. The requirement for Tat is eliminated due to the use of a CMV-LTR fusion promoter for vector RNA production. The risk of RCR generation from such transfection is significantly minimized due to two reasons; (1) since the genes required for vector production are included in several different plasmids, the potential of generating RCR through DNA recombination during transfection or through RNA co-packaging is much reduced; (2) since Tat is an essential function for HIV replication, the absence of this gene during vector production makes it unlikely for RCR production. One concern is that four plasmid co-transfection may generate poor vector titers. However, reports from various labs suggest the feasibility of producing high vector titers with such an approach. It is not clear why the investigator did not adopt such a system for vector production. One other question is why the investigator uses 293 instead of 293T cells for vector production. Besides the expression of SV40 T antigen in 293T cells that allows replication of SV40 origin-containing plasmid, 293T cells actually have higher transfection efficiency than 293 cells. Vectors prepared from 293T cells may have better titers than that from 293 cells.

3. The single most critical issue for HIV vector application is to establish sensitive assays for RCR detection. Recombination between the gag/pol genes in the helper construct and the gene transfer vector has been detected by a marker rescue assay (Mol. Ther. 2: 47-55). Such recombinants can transfer the HIV gag and pol genes into target cells, and can be mobilized by wt HIV infection and spread further. While such event remains a concern for gene therapy in non-HIV diseases, it may be a lesser problem for HIV gene therapy since patients have already been infected with HIV. But this observation does illustrate how easy it is to generate recombinants with the current 293 co-transfection protocols. Assembly of all the required genes for vector production in a single plasmid construct as described in the current application would certainly increase the likelihood of such a recombination event. One additional recombination that results in the insertion of the VSV-G gene would create a recombinant virus with broad host range. In this reviewer's opinion, it is important to set up a marker rescue assay as described by Wu et al. (Mol. Ther. 2:47-55) to evaluate the recombination event described above. If such an event indeed occurs, the recombinant may not be detected with the proposed p24 or RT-PCR assay, depending on whether the recombinant also contains the Tat and Rev genes. Infection of wt HIV as is the case in HIV patients, however, can mobilize such uncharacterized vectors and facilitate their spread. More importantly, VSV-G expression may be activated by wt HIV infection, resulting in the potential pseudotyping of wt HIV with VSV-G.

4. In Table 7, the proposed RCR assay to release viral vector will be performed in 293 cells. However, in the latter section, H9 cells were used for vector release RCR test (page 22, Tables 9 & 10). Why is such a discrepancy? Is H9 the most sensitive cell line for HIV replication and p24 detection? To enhance the sensitivity of RCR detection, is it necessary to screen a number of commonly available human hematopoietic cell lines that may permit even more efficient HIV replication (as judged by the amount of p24 produced) than 293 or H9 cells?

5. The persistence of transduced T lymphocytes in vivo is partly dependent on the patient's viral load. To facilitate data evaluation, is it important to select study population with specific ranges of viral load?

6. Both Bukovsky et al. (J. Virol. 73: 7087-7092) And Evans and Garcia (Hum. Gene Ther. 11: 2331-2339) reported the mobilization of HIV vectors by wt HIV infection. This, however, did not occur in the current application as shown in Fig. 19. Could the investigator use too much wt HIV for challenge that competes with the rescued vector for infecting naïve Sup T1 cells? If vector mobilization is not observed, what is the advantage of using the proposed vector? Is it safer to use third-generation HIV vectors from which most of the cis-regulatory elements in the U3 region were removed and the requirement for Tat was eliminated?

7. For vector releasing testing as shown in Fig. 20, is it possible to co-cultivate the transfected 293 cells with H9 cells for 6 passages? Since VSV-G expression will

most likely cause cytotoxicity to the transfected 293 cells within a few days, it may be difficult to co-cultivate the two cell lines for that many passages. How effective is the Benzonase treatment to remove contaminating DNA from the vector preparation? If the TaqMan assay is positive for VSV-G sequence, what is the criteria for vector release?

8. The investigator concludes that since the biological assay for RCR is negative, the VSV-G DNA detected by the TaqMan assay in the transduced cells cannot be derived from RCR. What then is the explanation for the detection of the VSV-G sequence? If the samples are isolated from cells of the 6^{th} passage, it is unlikely that the residue contaminating plasmid DNA is still present at that point. As explained above, vectors derived from recombination events may contain the VSV-G gene. Cells transduced with such vectors would be positive for VSV-G DNA by PCR, but negative for the p24 assay if they do not also contain the Gag/Pol, Tat and Rev genes. Thus, assays need to be established to detect such recombinants.

9. To evaluate potential dissemination of the vector, the investigator proposes to study bio-distribution of the vector in SCID mice. One major problem of using mice for such studies is that multiple blocks for HIV replication exist in mice. Even if the RCR-rescued vectors overcome the cell entry problem due to the VSV-G protein, the absence of cyclin T1 and inefficient gag processing in murine cells would make this assay very insensitive. Face with these problems, should mice be used as the animal model to evaluate potential vector dissemination?